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Seasonal variation in the abundance and distribution of ticks that parasitize *Microcebus griseorufus* at the Bezà Mahafaly Special Reserve, Madagascar

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ABSTRACT

At Bezà Mahafaly Special Reserve (BMSR), Madagascar, mouse lemurs (*Microcebus griseorufus*) are parasitized by multiple species of haemaphysaline ticks. At present we know little about the role ticks play in wild lemur populations and how they can alter interspecies relationships within communities or impact host fitness. In order to better understand these dynamics at BMSR, we examined parasite-host interactions as well as the ecology of mouse lemurs and their infesting ticks, *Haemaphysalis lemuris* and *H.* sp. cf. *simplex*. We show that season, host sex, and habitat influence the relative abundance of ticks on mouse lemurs. Specifically, infestations occur only during the dry season (May–October), are higher in males, and are higher at the study site with the most ground cover and with greater density of large-bodied hosts. *Microcebus* likely experience decreased susceptibility to tick infestations during the wet season because at that time they rarely if ever descend to the ground. Similarly, male mouse lemurs have higher infestation rates than females because of the greater time they spend traveling and foraging on the ground. During the dry season, *Microcebus* likely serve as hosts for the tenrec tick, *H.* sp. cf. *simplex*, when tenrecs hibernate. In turn, during the wet season when mouse lemurs rarely descend to the ground, other small mammals at the reserve may serve as maintenance hosts for populations of immature ticks. The synchronous development of larvae and nymphs could present high risk for vector-borne disease in *Microcebus*. This study also provides a preliminary description of the ecology and life cycle of the most common lemur tick, *H. lemuris*.

1. Introduction

Epidemiological studies of ectoparasitism in lemurs have generally focused on diagnostics (Takahata et al., 1998; Junge, 2002; Loudon et al., 2006; Durden et al., 2010). Few have provided significant information regarding parasite-host interactions or the ecology of the parasites. Understanding host-parasite relationships and tick ecology is important for evaluating the hosts' risk of disease from ticks or from microparasites that ticks may carry; this in turn can be critical for conservation management. Wild *Microcebus* (mouse lemurs) live in relatively high densities, often descend to the ground, and engage in social grooming. These characteristics place them at high risk for ectoparasite infestation. In fact, mouse lemurs are parasitized by multiple species of ticks. These small primates primarily present immature tick stages (Durden et al., 2010; Rodriguez et al., 2012; Blanco et al., 2013) and likely serve as maintenance hosts to various three-host tick species, including *Haemaphysalis lemuris*, *Ixodes lemuris* (Blanco et al., 2013), and other *Haemaphysalis* spp. (Durden et al., 2010; Rodriguez et al., 2012).

At the Bezà Mahafaly Special Reserve (BMSR) in southwestern Madagascar *Microcebus griseorufus* are parasitized by *Haemaphysalis lemuris* and another tick, possibly *Haemaphysalis simplex* (Rodriguez et al., 2012), and which we call here conservatively *H.* sp. cf. *simplex*. *Haemaphysalis lemuris* is the most common lemur tick although little is known about its life cycle. This tick has been collected from at least nine lemur species (Hoogstraal and Theiler, 1959; Koyama et al., 2008; Durden et al., 2010; Junge et al., 2011),

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including two larger-bodied lemur species, *Propithecus verreauxi* (Verreaux' sifakas) and *Lemur catta* (ring-tailed lemurs), that live in sympatry with *M. griseorufus* at BMSR (Takahata et al., 1998; Loudon et al., 2006; Loudon, 2009). The second tick species exhibits morphological characteristics similar to the old world *Haemaphysalis* subgenus specialized for parasitizing birds as well as tenrecs, *Ornithophysalis* (e.g., *H.* (*Ornithophysalis*) *simplex* and *H.* (*O.*) *simplicima*) (Hoogstraal, 1953; Hoogstraal et al., 1974). At Ranomafana, Durden et al. (2010) observed haemaphysaline ticks (*Haemaphysalis* sp.) on *Microcebus rufus* that could not be identified. Unfortunately, no description of *Haemaphysalis* sp. was provided and it is therefore not yet possible to confirm the species status of the second haemaphysaline tick found at BMSR. While more work is necessary to verify species identification, nymphs of the tick species collected from mouse lemurs at BMSR have tentatively been identified as *H. simplex* by morphological analysis (Rodriguez et al., 2012).

At BMSR, tick infestations on mouse lemurs are not random; instead, both *Haemaphysalis lemuris* and *H.* sp. cf. *simplex* are found on mouse lemurs exclusively during the austral winter and primarily at one of the reserve's two "parcels," which are non-contiguous forests (Rodriguez et al., 2012). Ticks have been recovered, however, from ring-tailed lemurs and sifaka from both of the reserve's parcels and at varying times of the year (Loudon et al., 2006; Loudon, 2009). Because mouse lemur infestations are restricted temporally and spatially, we believe that patterns of parasitism at the reserve are influenced by the life cycles of parasitizing ticks and the ecology of the hosts. In addition, the presence of *H.* sp. cf. *simplex* on mouse lemurs at BMSR indicates that mouse lemurs serve as alternate hosts to ticks from other mammalian species.

Here we examine tick infestations of *M. griseorufus* in their ecological contexts at and in the vicinity of the Bezà Mahafaly Special Reserve to determine which factors likely control haemaphysaline tick abundance and distribution. We address these questions by exploring infestation rates of ticks on *M. griseorufus* males and females living in different microhabitats and within the same microhabitat at different times of the year. We test three hypotheses (that habitat matters, that sex matters, and that season matters) and examine mouse lemur behavioral characteristics that may affect their tick infestation rates and their potential as hosts to various tick species. Finally, on the basis of this information we present a preliminary description of the ecology and life cycle of *H. lemuris*.

2. Materials and methods

2.1. Study sites

Microcebus living in three non-contiguous forests were studied for tick infestations; two inside the Bezà Mahafaly Special Reserve (Parcels 1 and 2) and one outside of the reserve (Ihazoara forest). Parcel 1 is an 80 ha gallery forest that is protected by a fence and is regularly monitored (Ratsirarson, 2003; Rasoazanabary, 2011). It borders a research camp. This site has considerable understory and thick ground litter. It contains the highest population densities of species of lemurs present at BMSR, *L. catta*, *P. verreauxi*, *Lepilemur petteri* (sportive lemurs) and *M. griseorufus*. Parcel 1 also has the highest density of the introduced rodent, *Rattus rattus* (Youssouf Jacky and Rasoazanabary, 2008). Parcel 2, is a larger 520 ha forest that is characterized by deciduous and Didiereaceae-dominated spiny vegetation (Ratsirarson, 2003; Axel and Maurer, 2011). The ground cover at Parcel 2 is much thinner than at Parcel 1. Ring-tailed lemurs are rare in Parcel 2; sifakas and sportive lemurs are more common. The third study site, in the Ihazoara forest, lies adjacent to Ihazoara village and is the most disturbed of the three sites. Livestock roam regularly through the site along paths created by the villagers. The vegetation is similar to that of Parcel 1, and the forest floor is rocky and virtually devoid of herbaceous vegetation (Rasoazanabary, 2011). At the study site, no ring-tailed lemurs or sifakas were observed. Fieldwork was conducted by ER.

2.2. Mouse lemur trapping

We used Sherman traps baited with banana to capture mouse lemurs during a year-long study (October 2006–September 2007). At each of our three study sites, we conducted intensive sampling in a large main study area (275 m × 225 m) during four months of the year (January, May, September and October). In addition, smaller or "supplementary" areas (20 m × 20 m) near the main study areas were selected for sampling during the other eight months of the year. In Parcel 1, the main study area was regularly used by researchers and a trail grid laid by prior researchers was used for this study. The supplementary study site was more pristine, with tall grass and leaf litter, as it was not regularly used by prior researchers and had no trail grid. A full description of the trapping schedule is provided by Youssouf Jacky and Rasoazanabary (2008).

We set traps in trees and on the ground at night and checked them each morning for captured animals. We marked captured mouse lemurs by clipping the ears, and inserting microchips for easy identification using a transponder. We collected basic data (date, place of capture, sex, and basic morphometrics including body mass) for each captured individual. Animals were released at the location of their capture around sundown, the beginning of their active period. On a daily basis, total rainfall and minimum and maximum temperature at Parcel 1 were also recorded.

2.3. Tick recovery and identification

All captured mouse lemurs were examined for ectoparasites and when present, all ticks were removed from the host and counted. For identification and future analysis, ectoparasites from 20 host animals were preserved in 70% ethanol or EDTA. Identification of *Haemaphysalis lemuris* and *H.* sp. cf. *simplex* ticks was made by comparing the nymphal ticks collected with those described previously (Hoogstraal, 1953; Uilenberg et al., 1979; Takahata et al., 1998), and by consulting with experts in the field. Morphological descriptions and images of both *Haemaphysalis* types are provided in Rodriguez et al. (2012). No voucher specimens of ticks were deposited in collections because all samples were utilized for genetic analysis and samples were destroyed during the DNA extraction process. Insufficient DNA was recovered from samples for amplification.

2.4. Statistical methods

We used the chi-square functions in Graph Pad Prism and the Statistical Package for the Social Sciences (SPSS 22.0) to ascertain the significance of differences in tick infestation rate by season, site and sex. A number of mouse lemur individuals were "trap happy" (captured multiple times – up to 43); some were heavily infested. Because these individuals become overrepresented when the sample comprises total captures and recaptures, comparisons by capture and recapture are useful only when looking at the overall infestation pattern across forest types and habitats. Comparisons by individual give a more accurate measure of infestation rates. For each statistical comparison, we indicate whether the test is based on number of captures or number of individuals in each test category.